

Microbial analysis and weight estimation of feces produced by four sympatric Collembola species in forest litter

BY

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INTRODUCTION

It is commonly admitted that most springtails feed mainly on fungi and bacteria and occasionally on pollen and other decomposing plant debris. The first precise observation was a photograph showing many spores in the digestive tract of an *Achorutes* (MAC NAMARA, 1924). The analysis of gut content through the previously cleared or dissected body, or of the feces, were further frequently used to study the various components of the diet in *Collembola*. Surface dwelling species (of the genera *Orchesella* and *Tomocerus*) feed mainly on fungal mycelia and a dozen types of food could be listed: spores, hyphae, plant membranes, parenchyma, tracheae, pollen, chitin, algae, organs such as bristles, scales, ecdysis, crystals, amorphous elements (BERNARDI and PARISI, 1968).

The bulk of the diet is made of fungi and the ecological consequence is that *Collembola* are usually more abundant in acid, mycelium-rich soils (KÜHNELT, 1963).

Strictly uniform diets do not seem to be known in *Collembola*. In field conditions, *Tomocerus flavescens* eats in preference fungal hyphae, while kept in culture, it rather chooses spores (KNIGHT and ANGEL, 1967). Although more spores than hyphae are usually found in feces, one may think that the

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latter are more easily digested and that spores, pollen grains, exuvia and plant debris are less important in the diet of Collembola (BÖDVARSSON, 1970). On the other hand, mineral particles mixed with other organic debris are more abundant in soil-dwelling than in surface-dwelling species. Such mineral particles may have an important nutritional value when they are clay aggregates wrapping up bacteria and organic matter (MAC MILLAN, 1975).

During an overall year cycle, the gut content of three *Entomobrya* species exhibited a seasonal variation in the relative importance of the amounts of fungi and humus: in autumn, *Tomocerus* ingested more fungi than *Orchesella* did (ANDERSON and HEALEY, 1972). A comparison of Tomoceridae of different biotopes showed that individuals living within a thick litter layer fed on humus and hyphae, while those living over a soil covered with a sparse litter mainly fed on spores and undetermined organic matter (GILMORE and RAFFENSPERGER, 1970). However, it is uneasy to state whether Collembola are more sensitive to the amount or to the quality of nutriment. For instance, the following experiment was significant: the gut content of three Onychiuridae living near an ant nest was compared to the content of glass microtubes considered as artificial guts and buried at random in the same area; no significant differences were marked and this result leads to suppose that Collembola do not choose their food (MAC MILLAN, 1975).

Seeing that it is not possible to determine the fungal species ingested by Collembola in nature, some authors have kept Collembola species on pure cultures of fungi and yeasts to detect feeding preferences. While the identification of fungi species in the gut mounted on microscopic slides is inconvenient, it is easier to make inoculations on nutritive media so as to isolate and determine the fungal species involved (KNIGHT and ANGEL, 1967). Such a method allows to draw a list of fungal species, not only within gut content, but also on exuvia and cuticle, from trails left by individuals and on excreted matter itself (CHRISTEN, 1975).

This concise survey of the main works dealing with the diet of Collembola reveals firstly that a quantitative study of the ingested food has not yet been tried and secondly that no objective method has permitted the study of composition and fine structure of matters after their transit in the digestive tract. The present study was undertaken to bridge this gap, with the aid of accurate techniques such as microgravimetry and electron microscopy.

I. — MATERIAL AND METHODS

The present study is performed on four Collembola species whose individuals live either on soil surface, or in the herbaceous stratum (*Mercurialis*) under the cover of a hornbeam wood in the park of the laboratoire d'Ecologie générale at Brunoy (Essonne, France).

Tomocerus minor is a hygrophilous species which most often lives hidden under dead leaves and twigs covering the soil. *Orchesella villosa* and *Orchesella cincta* are two sympatric *sensu stricto* species which are frequently occurred over the surface of plant debris, immobile and directly exposed to turbulent air currents.

Allacma fusca is a sturdy globular species able to leave the litter and to explore herbaceous stratum. These positions are modified according to microclimatic variations and

the four species may frequently be found together for long periods. They seem to share the same food resources, and the present study is carried out to confirm this hypothesis by examining their dejections.

Individuals of the four species were collected with a mouth aspirator on a few square meters in the field on the same day, in september 1977. A first lot composed of a score of individuals was placed on wet filter paper for the recovery of fecal pellets which were immediately fixed for TEM (fixation: RYTER and KELLENBERGER, 1958; Staining: REYNOLDS, 1963; THIERY, 1967) or SEM (Critical point fixation). A second lot of the same importance was used to calculate fresh body weight, dry body weight and unfatted dry body weight of each specimen just after sampling. A third lot was deprived of food so as to determine their biomass with empty gut. The weight data allowed a statistical analysis of the amounts of food ingested by *Collembola*. An automatic electrobalance (Cahn, 4700) was used to weigh each animal within one microgram resolution.

II. — ANALYSIS OF FECES IN ELECTRON MICROSCOPY

In scanning electron microscopy, fecal pellets of *Tomocerus minor* appear mainly made of strangled hyphae (Pl. I, 1). These hyphae are often intact, with a smooth surface and constant diameter (Pl. I, 2). Some of them shows cut ends. Fungal spores never occur on the surface of feces.

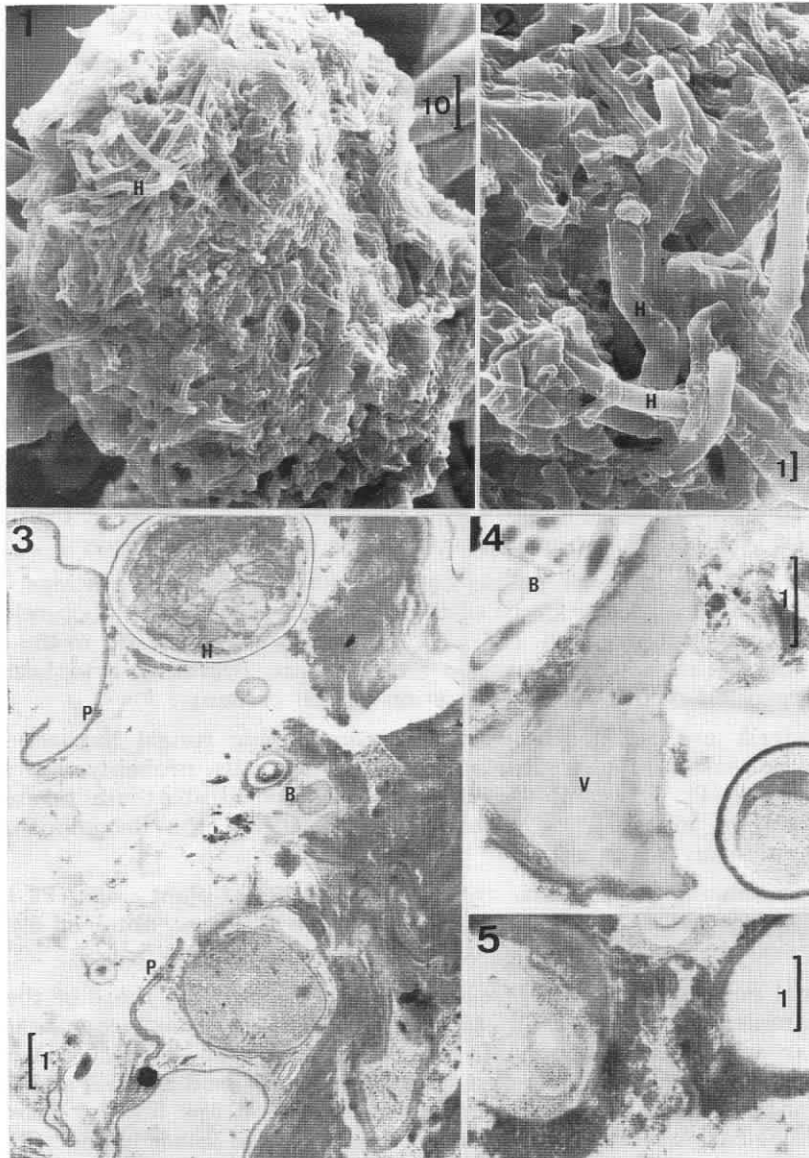
Thin sections through pellets confirm the previous observations. Many fungal hyphae are present, most of them with a normal ultrastructural aspect and cellular components (Pl. I, 3). *T. minor* may therefore aid in the dispersal of these fungi. Other hyphae have a more degraded cytoplasm, and walls are often recorded after digestion of cell contents.

Bacteria are frequently present accompanying fungal hyphae, in the vicinity of masses of biological membranes and walls, probably the remains of digestion (Pl. I, 3 and 4). Bacteria often penetrate these masses and produce lysis zones. They probably participate in the decomposition of nutriments even in the gut.

Occasionally other debris are visible: remains of plant walls (Pl. I, 4) or fungal spores (Pl. I, 5). These results agree with those obtained by other workers on the same species or other species of the same genus.

Fecal pellets of *Allacma fusca* are slightly longer than those of the other species under study (Pl. II, 6). Mounted on SEM their surface appears very irregular and their components are compacted and hardly recognizable. Sometimes however, an hypha or a fungal spore is noticed (Pl. II, 7 and 9). The appearance in TEM is very homogenous. Besides a few fibrillar debris and rare biological membranes, the main components are intact hyphae (Pl. II, 8) and deeply altered spores (Pl. II, 11). In the latter, cytoplasm is usually digested and the three different layers of the cell wall tend to be separated: an external fibrillar layer, an intermediate layer also fibrillar but more compacted and polysaccharide rich (Pl. II, 10) and an internal granular layer. No bacteria occurs in feces of this surface dwelling species.

Fecal pellets of *Orchesella villosa* in SEM show mycelial fragments which seem to be devoid of any content (Pl. III, 12). The main components of the feces were globular elements, probably fungal spores or algae. Sections



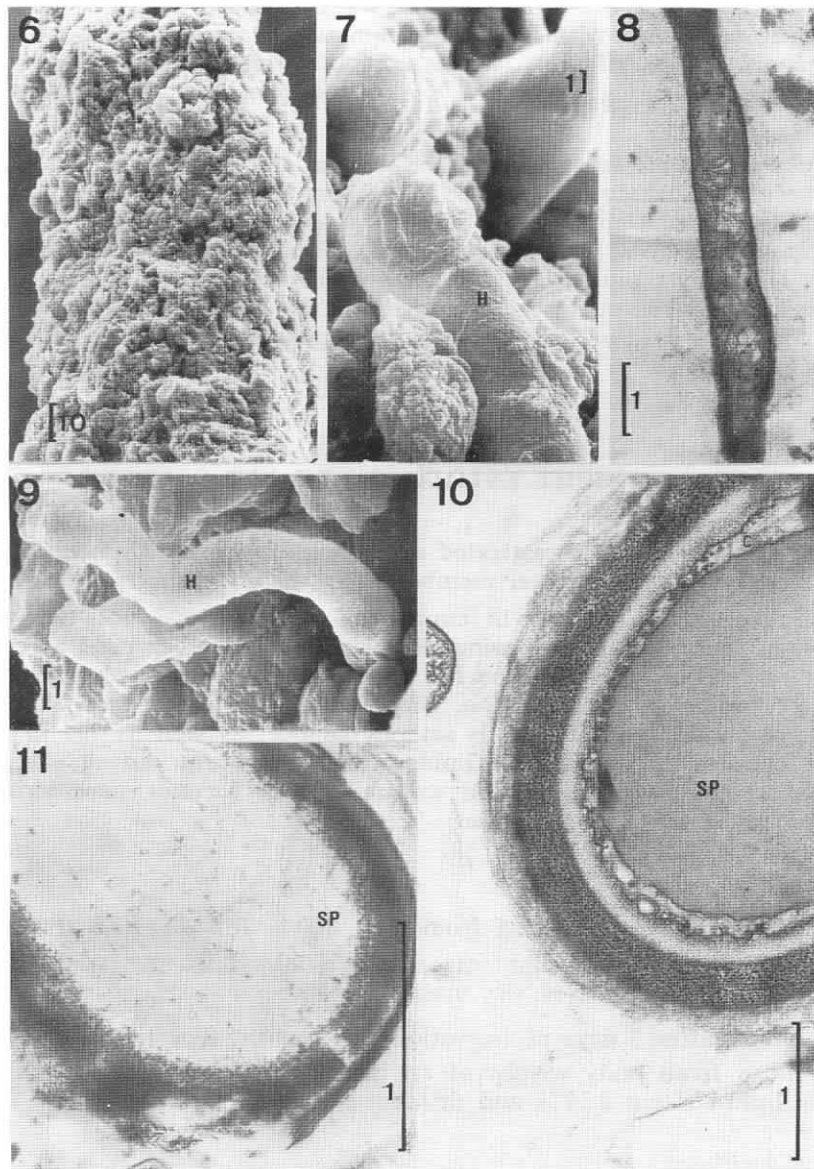
PL. I. — *Tomocerus minor* (Lubbock). Scale marked in μm .

1 and 2 : Fecal pellet wrapped in fungal hyphae.

3 : Thin section exhibiting hyphae (H), Bacteria (B) and wall debris (P). Reynolds staining.

4 : Bacteria (B) close to plant wall debris (V). Reynolds staining.

5 : Remains of fungal spores. Reynolds staining.



PL. II. — *Allacma fusca* (Linné). Scale marker in μm .

6, 7 and 9 : Fecal pellet coated with hyphae (H).

8 : Viable hyphae. Reynolds staining.

10 : Fungal spore (SP) with three wall layers. The middle layer is rich in polysaccharides. Thiery's technique.

11 : Deeply altered fungal spore (SP) with fibrillar external layer and a granular internal layer. Reynolds staining.

in TEM show barely modified fungal hyphae (Pl. III, 14). Sometimes intra-hyphal hyphae are seen. Algal walls are not contrasted with lead citrate but are getting very dark with Thiéry's technique, revealing the abundance of polysaccharides (Pl. III, 13). Walls seem little altered but cytoplasm has disappeared, indicating an active use of cell content. Bacteria are not present.

Orchesella cincta fecal pellets are spherical and microorganisms are only rarely seen on their surface (Pl. IV, 15). Many spores are present, and most of them are broken; this finding suggests that the inner part of spores has been digested (Pl. IV, 16). Feces seen in TEM contain many intact algae (Pl. IV, 17). Thylakoids, lipid globules are visible in cell content. In some instances, these structures were degraded, but the cell content is only partially used. Many bacteria are recorded in these pellets, mainly close to organic debris (Pl. IV, 18).

III. — WEIGHT ESTIMATION OF GUT CONTENT

This study has been investigated on *Allacma fusca* and *Orchesella villosa* which were collected in greater numbers than the other species.

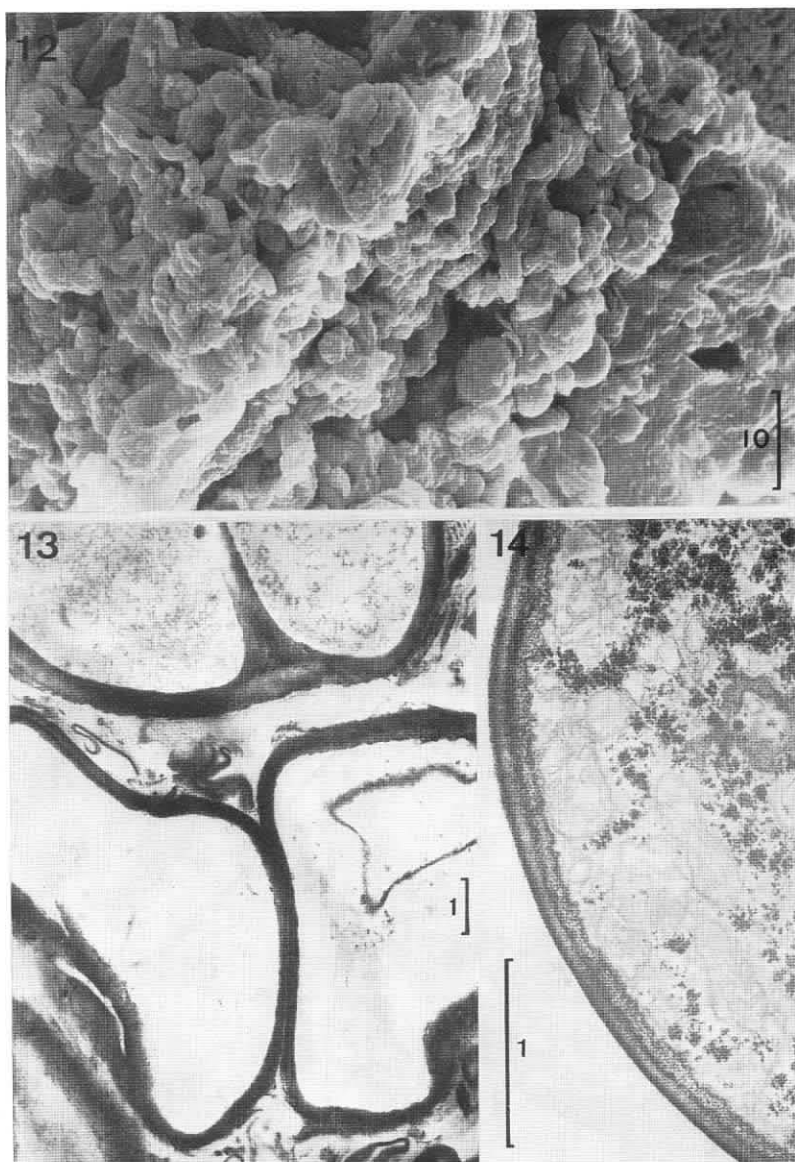
The experiment consists in comparing the biomasses of two groups ($n = 24$) of individuals, one having been starved, the other used as control. Then it was necessary to choose the least affected weight value by physiological consequences of starvation. The fresh body weight was eliminated, because the water equilibrium is set off balance, as well as the dry body weight, because it contains the lipids which are burnt during starvation; only the weight after delipidation was taken into account, considering the lipid content of feces as negligible.

Allacma fusca.

1. — Control, animals collected from field, 100 % full digestive tracts.
— mean fresh body weight of an individual = 2.398 mg; water content 76.10 %, lipid content 5.25 %, dry matter 19.64 %.
2. — Animals after 3 days of starvation, 96 % empty digestive tracts.
— mean fresh body weight of an individual: 2.596 mg; water content 80.74 %, lipid content 2.54 % and dry matter 16.21 %.

Orchesella villosa.

1. — Control, animals collected from field, 66 % full digestive tracts.
— mean fresh body weight of an individual = 2.045 mg; water content 73.84 %, lipid content 5.87 %, dry matter 20.29 %.
2. — Animals after 8 days of starvation, 100 % empty digestive tracts.
— mean fresh body weight of an individual = 1.697 mg; water content 81.14 %, lipid content 2.65 % and dry matter 16.21 %.

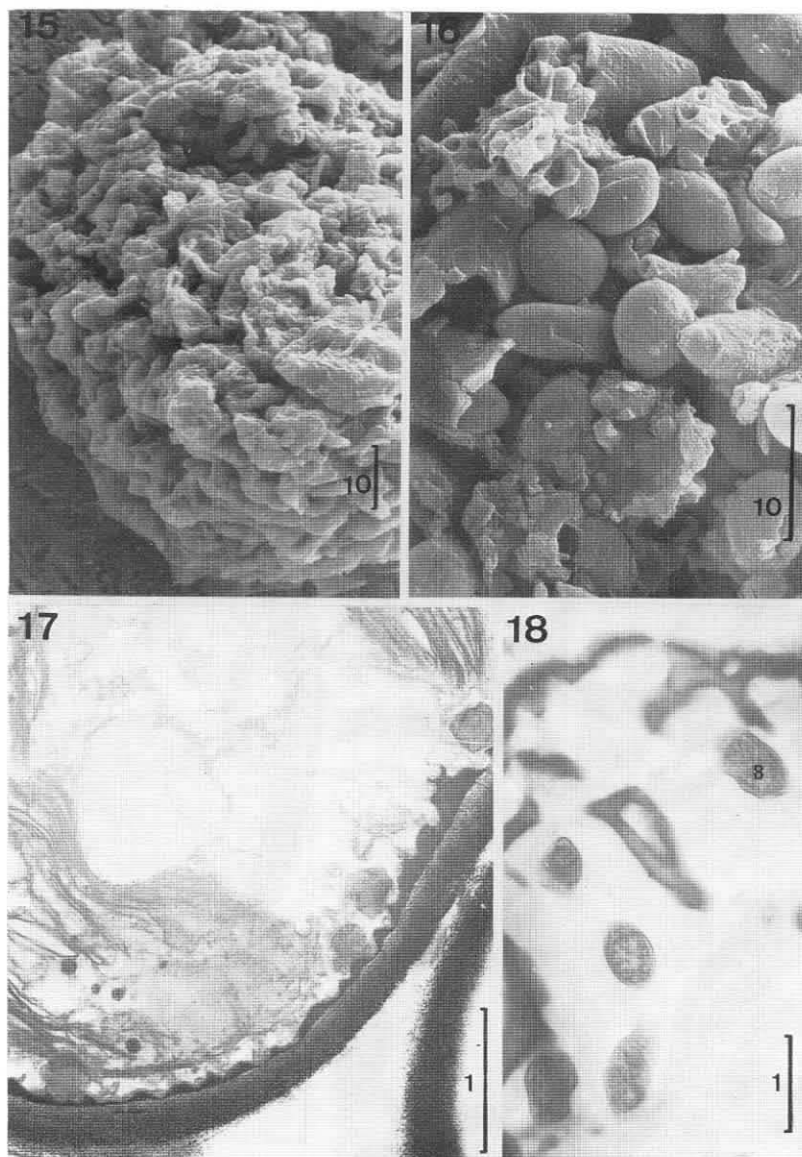


PL. III. — *Orchesella villosa* (Geoffroy). Scale marked in μm .

12 : Fecal pellet made up of hyphae and globular elements which may be fungal spores or algae.

13 : Algae with spent cytoplasmic contents. Thiery's technique.

14 : Active fungal hyphae. Thiery's technique.



PL. IV. — *Orchesella cincia* (Linné). Scale marked in μm .

15 : Fecal pellet with irregular elements incorporated.

16 : Broken spores on the surface of pellet.

17 : Viable algae; polysaccharide-rich wall, Thylakoids and active photosynthetic lamellae. Thiery's technique.

18 : Bacteria (B) close to organic debris. Reynolds staining.

Figure 1 shows regression lines settled for both species, between animals with a full digestive tract and those without gut content. For a P_3 (third leg) claw length L , which is considered as a good size index, the difference ΔZ (mg) between two regression lines indicates the dry weight of gut matter in a Collembola.

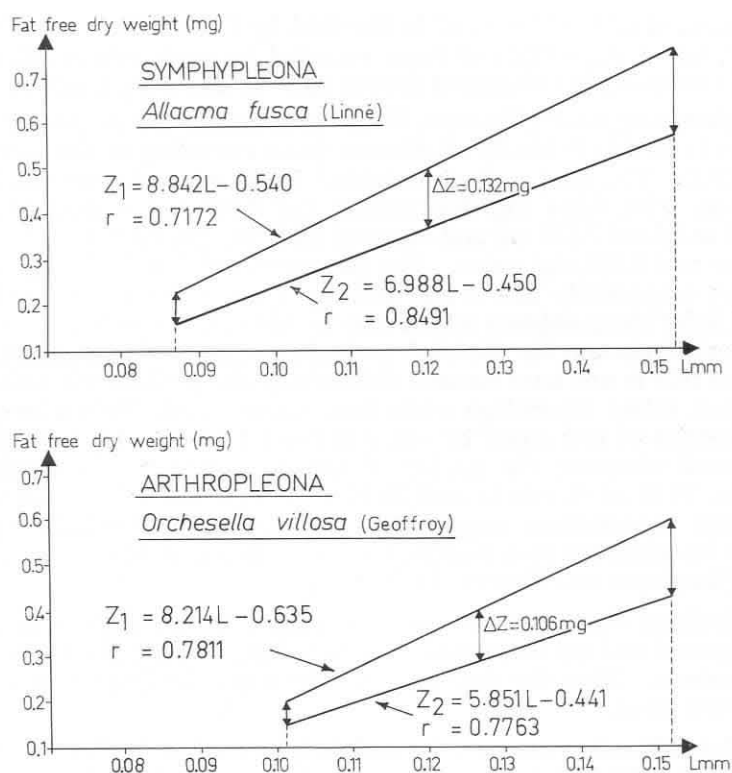


FIG. 1. — Statistical weight estimation of gut content in individuals of two coexisting populations of Collembola. L : claw length of third leg; Z_1 : linear regression on population collected from field; Z_2 : linear regression on population deprived of food; ΔZ : mass of individual gut content; r : correlation coefficient.

For *Allacma fusca* $Z = 1.854 L - 0.090$

an animal with a 0.120 mm claw has a dry body weight of 0.661 mg; the dry mass of its gut content is 0.132 mg or 20 % of its dry body weight.

For *Orchesella villosa* $Z = 2.363 L - 0.194$

an animal with a 0.127 mm claw has a dry body weight of 0.526 mg; the dry mass of its gut content is 0.106 mg or 20.2 % of its dry body weight.

The weight of food ingested by Collembola is enormous compared to some Orthoptera which are known as voracious herbivores. A first instar

larva of *Myrmeleotettix maculatus*, feeding on gramineous leaves, weighs 1.536 mg and produces only 0.132 mg dry feces, or 8.6 % of its dry body weight.

IV. — CONCLUSION

The amount of food ingested in the field by Collembola seems to be very important, when the weight of feces excreted by each animal after a short period of starvation is considered (about 20 % of dry body weight).

In a previous paper (VANNIER, 1973) we reported the results of recorded water loss in two individuals of *Allacma fusca* excreting at the same time a dozen pellets. The first animal weighted 1.060 mg and excreted 0.131 mg humid feces with 0.064 mg dry matter and 0.067 mg water. The second individual weighted 3.840 mg and excreted 0.407 mg humid feces with 0.184 mg dry matter and 0.223 mg water. The percentage of humid feces/fresh body weight was respectively 12.4 % and 10.6 %. These rates are rather high and one could infer these animals which may be present in number of thousands/m², are more important for the biodegradation process of primary production. But in fact this is not true because Collembola are periodically submitted to physiological crises (moulting) while they cannot feed. This was confirmed in three species of our study by other authors (WITH and JOOSSE, 1971) who demonstrated whatever the period of the year 62 % of *Orchesella villosa* individuals, 75 % of *O. cincta* and 58 % of *Tomocerus minor* had an empty gut. Similar observations suggest that the average Collembolan spends 40 to 50 % of its adult life in a inactive or semi-active condition in which it does not feed (ANDERSON and HEALEY, 1972).

Our findings show that all food consumed by Collembola is not completely digested and an important part is rejected under the form of viable microorganisms. Then the role of Collembola may be important in the dispersal of soil fungi.

The diet of *T. minor* seems to be mainly composed of hyphae, most of them are empty, as exhibited by the presence of abundant fungal walls. *A. fusca* also feeds preferentially on fungi, but spores are digested while hyphae appear intact. *O. villosa* mainly feeds on algae, while *O. cincta* ingested algae too, but only uses a small part of their cytoplasmic content. Part of the matter ingested by these two sympatric species is identical, but the degradation process of food is quite different. This hypothesis is confirmed by the abundance of bacteria in the feces of *O. cincta*.

These preliminary results indicate that the four Collembola species share the food resources of a restricted, apparently homogenous biotope, and do not compete for food supply according to the law of interspecific coexistence (de BACH, 1966). Such qualitative and quantitative analysis are obviously valid only for a given biotope and a given period, since the diet of animals may vary with the availability of food in the sampling area.

Such a method of quantitative and qualitative analysis is used for the first time on Collembola collected in nature. Observations in transmission electron microscopy are determinant to analyse objectively Microarthropod

diets, by evidencing food particles decayed by digestive enzymes. As far as we know, this method had previously been applied only to Acarina (*Tyrophagus putrescentiae*) but in laboratory experiments (REISINGER, 1973). Now we are planning to carry out observations of thin sections across different parts of the digestive tract, so as to follow the main steps of digestion in animals collected in nature or raised in the laboratory on selected microbiological culture media.

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SUMMARY

A qualitative and quantitative analysis of diet in four Collembola species inhabiting the same forest litter has been investigated using fine techniques such as electron microscopy and microgravimetry.

A comparative study of feces content on scanning and transmission electron microscopes showed that species share the food resources in the same biotope (spores, fungal hyphae, algae and bacteria) and do not compete for food supply. These results are in accordance with the general ecological rule of interspecific coexistence.

The amount of food ingested by these minute insects seems very important when considering the weight of feces rejected by each individual, about 20 % of its dry body weight after a three or eight days period of starvation.

RÉSUMÉ

L'utilisation de techniques fines en microscopie électronique et en microgravimétrie a permis d'entreprendre une étude quantitative et qualitative du régime alimentaire de quatre espèces de Collemboles vivant dans la litière de forêt.

L'analyse comparée des fèces en microscopie électronique à balayage et à transmission a révélé que les quatre espèces se partagent les ressources alimentaires dans le même biotope (spores, hyphes de Champignons, algues et bactéries) et n'entrent pas en concurrence trophique, respectant ainsi la règle générale en écologie de la coexistence interspécifique.

Les quantités de nourriture absorbées par ces petits insectes semblent très importantes si on s'en réfère à la masse de matière fécale rejetée par chaque individu, environ 20 % de son poids sec corporel, après une période de 3 ou 8 jours de jeûne.

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